National Institute for Occupational Safety and Health

Top Priorities

NIOSH has three priorities in the area of genetics.

1. Conduct of research on the interaction of occupational exposures and genetics factors in the development of disease.

2. Use of genetics markers to assess health effects of occupational exposures

3. Development of guidance information for the use of genetics in occupational safety and health

Gene environment interactions
Despite the strong causal associations that have been detected in many occupational studies, there are differences in disease incidence between groups of workers that cannot be accounted for by differences in exposures, work practices, or lifestyle. Genetic polymorphisms are likely to be responsible for some of these differences. In the early history of occupational epidemiology, it was generally accepted that genetic influences were accounted for by controlling for confounding by race and gender. Today, as many occupational exposures are lowered, the importance of genetic information as a source of variability in risk estimates is increasing. This is not to imply that occupational etiologies will be replaced with genetic etiologies; rather, polymorphisms, which might modify exposure–disease associations, should be included as relevant variables in study design and analysis. Although the individual risk associated with a genetic polymorphism may be relatively low, the population-attributable risk may be large, thus indicating the public health importance of this research. Genetic research that evaluates the role of specific genes in occupational disease or injury contributes to understanding the mechanisms and helps to disentangle which causal factors may pose the greatest threats to workers. NIOSH has looked specifically at gene environment interactions for chronic beryllium disease, Parkinson disease, and brain cancer.

Gene-environment interactions in chronic beryllium disease are being investigated in a case control study of beryllium manufacturing workers. This study is superior to previous studies in its improved exposure assessment, its increased size and because it represents a longitudinal follow-up. Previous studies have identified certain common genetic variants of a major histocompatibility complex gene, HLA-DPB1, to be associated with susceptibility to chronic beryllium disease (CBD). Many research questions remain of the more than 100 known HLA-DPB-1 variants, precisely, which are associated with disease? Is the association with beryllium sensitization? Do some alleles covey greater risks than others? Is latency of CBD genetically determined? A sophisticated PCR-DNA sequencing assay has been developed to more precisely identify specific HLA-DPB-1 haplotypes. Worker education and case control recruitment is

proceeding in collaboration with DRDS. To date this project has identified certain HLA-DPB1 that are associated with CBD and beryllium sensitization, it has identified homozygotes of those variants to be at higher risk of disease than heterozygotes (OR = 3.1, 95%CI = 1.5-6.1). Molecular epidemiology, together with molecular modeling, appears to show that HLA-DPB1 alleles that code for HLA molecules with greatest negative charge (-9) convey significantly more risk than those coding for HLA molecules that are moderately negatively charged (-7) (OR = 3.2, 95%CI = 1.9-5.4). Worker education efforts continue, which includes a two-day meeting at NIOSH, Morgantown, where workers now participate in making presentations.

Brain cancer is the fourth leading cause of cancer death among middle-aged men in the United States, and incidence and mortality rates are steadily increasing. One plausible hypothesis for the increasing incidence is increased exposure to etiologic agents or exposure to newly introduced carcinogens, such as pesticides and other chemicals. Even though farmers experience a lower overall cancer mortality than the general population, several studies indicate that individuals working on a farm or in the agricultural industry have an excess risk of brain cancer. NIOSH is conducting a population-based case-control study to determine if specific exposures prevalent among farmers and people living near farms are associated with increased risk for developing primary intracranial gliomas and to identify genetic susceptibility factors associated with brain cancer. Employing DNA specimens from 325 cases and 579 controls, NIOSH has analyzed over 90 gene variants in genes potentially related to brain cancer, such as polymorphisms in genes involved in activation and detoxification metabolism, DNA repair, cell cycle control, cell-to-cell communication, and immune function. In 2004, NIOSH evaluated the associations of polymorphisms in genes important in estrogen metabolism with the risk of glioma in women, since gender differences in brain tumor incidence and an analysis of questionnaire-reported reproductive and hormonal data from the study population suggested that hormonal factors might play a role in the etiology of these tumors. NIOSH found that polymorphisms in estrogen metabolism genes did not appear to have a strong association with glioma risk in women, although they have been linked to susceptibility in other cancers in women

Health effects of toxic exposures

Genetic monitoring is the evaluation of an exposed population for genetic damage over time and involves the detection of biomarkers of the effect of exposure. Much of this has grown out of the effort to assess workers and populations exposed to nuclear weapons and nuclear medicine techniques. Somatic mutations, DNA and protein adducts, and other cytogenetic changes have frequently been used as biological measures of exposure and in some cases as biomarkers of effect. The evaluation of changes in genetic material is usually part of research studies that investigate the effects of exposure or can be part of periodic medical examinations performed specifically for genotoxic agents in the workplace. Specific studies conducted by NIOSH are described below.

A previous NIOSH study showed an increase in sister chromosome exchanges in hospital workers exposed to ethylene oxide, an IARC Group I carcinogen, as compared to unexposed hospital workers. NIOSH will reassess exposed and unexposed former

workers with baseline cytology for levels of chromosomal damage, and evaluate them for susceptibility markers (polymorphic metabolic & DNA repair genes).

1-Bromopropane (1-BP) is an alternative to ozone-depleting chlorofluorocarbons that has a variety of potential applications as a degreasing agent for metals and electronics, and as a solvent vehicle for spray adhesives. As part of two NIOSH Health Hazard Evaluations (HHEs), DNA damage was assessed in peripheral leukocytes from workers with occupational exposure to 1-BP. Start-of- and end-of-workweek blood and urine samples were collected from workers at two facilities where 1-BP was used as a solvent for spray adhesives in foam cushion fabrication. Urinary bromine (Br) levels served as a biomarker of exposure. The comet assay was used to estimate DNA damage. In 1-BP exposed workers, start-of- and end-of-workweek comet endpoints were stratified based on job classification. Data were analyzed by combining the data sets from both facilities, log transformation of 1-BP exposure indices, Pearson correlation analysis, and the use of multiple linear regression models for each combination of exposure index and the level of DNA damage. Pearson correlation analysis indicated DNA strand breaks were positively associated with urine ($r= 0.288$, $P= 0.026$) and serum ($r=0.259$, $P=0.044$) Br concentrations, but stratification of workers into exposure quartiles did not reveal any significant differences and supported the conclusion that workplace exposure to 1-BP was not associated with increased DNA damage in leukocytes at these two facilities

The use of genetic information in occupational health studies

Because of these scientific advances, genetics has begun to transform research questions and study designs in the applied sciences of public and occupational health. Genetic studies provide new ways to study "risks" by evaluating genes and gene–environment interactions. The incorporation of genetics into occupational safety and health research generally requires collecting biological specimens from participating workers, analyzing those specimens, and developing test and study results. High throughput technology, such as microarrays, presents a number of challenges in terms of validity, data reduction and summarization, and analysis and interpretation. Analysis of these large datasets will also amplify the challenges that already exist when trying to relate genetic information and environmental factors. NIOSH has begun a discussion of study design and data interpretation issues that affect occupational health studies. A guidance document is currently being developed to address genetic issues in the workplace.

Major Accomplishments, 2004

Gene-Environment Interactions

Eleven papers in the area of gene environment interactions have been published this year.

Snyder, J., Weston, A., Tinkle, S.S., Demchuk, E.: Electrostatic potential on human leukocyte antigen: implications for putative mechanism of chronic beryllium disease. *Environmental Health Perspectives,* 111: 1827-1834, 2003.

McCanlies, E.C., Ensey, J., Schuler, C., Kreiss, K., Weston, A.: The Association Between *HLA-DPB1*^{Glu69}, Chronic Beryllium Disease, and Beryllium Sensitization. *American Journal for Industrial Medicine,* 46: 95-103, 2004.

Keshava, C., McCanlies, E.C., and Weston, A.: *CYP3A4* Polymorphisms in breast and prostate Cancer: A HuGE Review. *American Journal of Epidemiology*, 160: 825-841, 2004.

Gwinn, M.R., Whipkey D. L., Weston, A.: the effect of oxythioquinox exposure on normal human mammary epithelial cell gene expression: A microarray analysis study. Environmental Health, 3: 9 – 19, 2004.

Keshava, C., Divi, R., Whipkey, D.L., Frye, B.L., McCanlies, E.C., Kuo, M., Poirier, M.C., and Weston A.: Induction of CYP1A1 and CYP1B1 and formation of carcinogen-DNA adducts in normal human mammary epithelial cells treated with benzo[a]pyrene. *Cancer Letters*, 2004, in press.

Keshava, C., Whipkey, D.L., and Weston, A.: Transcriptional signatures of environmentally relevant exposures in normal human mammary epithelial cells: benzo[a]pyrene. *Cancer Letters*, 2004, in press.

Mahadevan, B., Keshava, C., Musafia-Jeknic, T., Pecaj, A., Weston, A., Baird, W. M.: Altered gene expression patterns in MCF-7 cells induced by the urban dust particulate complex mixture SRM 1649a. *Cancer Research*, 2004, in press.

Weston, A., Snyder, J., McCanlies, E.C., Schuler, C. R., Kreiss, K., Demchuk, E.: Immunogenic factors in chronic beryllium disease. *Mutagenesis*, 2004, in press.

Tinkle, S.S., Weston, A.: Beryllium toxicity and disease. Encyclopedia of Toxicology, Springer-Verlag. Third Edition. 2004, in press.

Weston, A., Ensey, J. S., Frye, B.L.: DNA-Sequence Determination of a Novel HLA-DPB1 Allele, HLA-DPB1*0403. *DNA Sequence*, 2004, in press.

Yuan, B-Z., Jefferson, A.M., Popescu, N.C., Reynolds, S.H. (2004) Aberrant gene expression in human non-small cell lung carcinoma cells exposed to demethylating agent 5-aza-2'-deoxycytidine. Neoplasia 6 (4):412-419.

Health effects of toxic exposures

Toraason, M., Singh, N. and Lynch, D.W. 2004. DNA damage in human leukocytes induced in vitro by 1- or 2-bromopropane. Toxicological Sci. 78(S-1):31-32.

Weston, A., Poirier, M.C.: Carcinogen DNA-adducts and DNA Repair. Encyclopedia of Toxicology, Springer-Verlag. Third Edition. 2004, in press.

Hooven, L.A., Mahadevan, B., Keshava, C., Perira, C., Desai, D., Amin, S., Weston, A., Baird, W.M.: effects of suberoylanilide hydroxamic acid and trichostatin A on induction of cytochrome P450 enzymes and benzo[a]pyrene DNA adduct formation in human cells. *Bioorganic & Medicinal Chemistry Letters*, 2004, in press.

Clark, A.M., Reynolds, S.H., Anderson, M. Wiest, J.S. (2004) Mutational activation of the MAP3K8 protooncogene in lung cancer. Genes, Chrom. & Cancer 41:99-108.

The use of genetic information in occupational health studies

Three papers were published this year that discussed the use of genetic information in epidemiological studies and practice.

Schulte PA. Some implications of genetic biomarkers in occupational epidemiology and practice. Scan J Work Environ Health 30 (1): 71-79, 2004.

Vineis P, Schulte PA, Carreon T, Bailer AJ, Medvedovic M. Issues in design and analaysis of gene-environment interactions. In P Buffler, J Rice, R Baan, M Bird, P Boffeta (eds). Mechanisms of carcinogenesis. IARC, Lyon France. IARC Sci Publ 157: 417-36, 2004.

Schulte PA. Interpretation of genetic data for medical and public health uses. In G Arnason, S Nordal, V Arnason (eds.) Blood and Data: Ethical Legal and Social Aspects of Human Genetic Databases. University of Iceland Press , Reykjavik, 2004.

Future Directions

NIOSH is proposing to develop a Laboratory for Occupational Genomics (NIOSH-LOG). The objectives of the proposed NIOSH-LOG are to 1) build an occupational biological sample base (DNA, blood, buccal smears, paraffin blocks, other) with which to perform molecular epidemiological genetic association studies to identify genetic risk factors of occupational disease. Samples will be available for investigators within the laboratory as well as NIOSH and non-NIOSH investigators that develop an appropriate study design. 2) Foster partnerships with academia, industry, other government and international agencies. 3) Provide a framework for data analysis that will be available for NIOSH investigators, academics and regulatory agencies. 4) Explore ethical challenges surrounding the use of genetic data in occupational health research. The focus, initially, will be on diseases currently being evaluated, such as silicosis, asthma, chronic beryllium disease, AZT treatment for HIV, irritant dermatitis, genetic susceptibility to lung cancer, and deterioration of lung function in fire fighters.

Autism is a developmental disability that is characterized by repetitive behavior, impairment in reciprocal social interaction, and difficulty communicating with and intuiting the feelings of others. A diagnosis of autism is generally made between the ages of two and three when there is a noticeable delay in language skills. It occurs most often in boys, but occurs in all social, racial, and ethnic groups. The prevalence has been estimated at 15-34 per 10,000. Genetic and epidemiologic research have shed some light

on the etiology of the disease, but further research on the potential role of environmental and occupational factors is needed. In a proposed study an investigation of the relationship between parental occupation, as defined by a job exposure matrix, and autism will be assessed.

Future gene expression studies using ejaculate sperm may yield important mechanistic insights regarding the effects of toxicants on male fertility. A pilot methods development project to assess gene expression in human sperm is underway. The recent discovery of spermatozoal coding RNAs that are delivered to the oocyte on fertilization has generated hypotheses regarding the role of sperm gene expression in human fertility and the potential effects of toxicants on sperm gene expression. The aim of this project, entitled "The Feasibility of Examining Gene Expression in Sperm," is to develop NIOSH field and laboratory methods to evaluate sperm gene expression, enabling investigators to potentially incorporate this parameter in future fertility and toxicant studies.